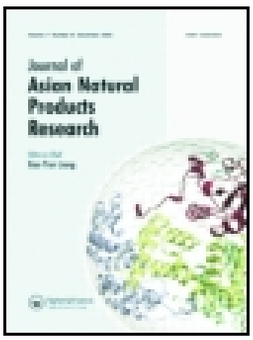

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Design and synthesis of 7-diethylaminocoumarin-based 1,3,4-oxadiazole derivatives with anti-acetylcholinesterase activities

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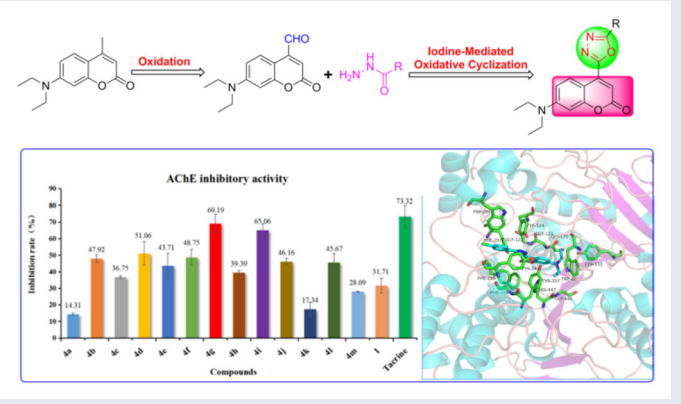
ABSTRACT

Twelve novel 7-diethylaminocoumarin-based 1,3,4-oxadiazole derivatives were synthesized via iodine-mediated oxidative cyclisation and confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. The result of these derivatives' activities inhibiting acetylcholinesterase *in vitro* showed that **4 g** and **4i** had moderate inhibitory activities with 69.19% and 65.06%, respectively. The preliminary structure-activity relationships revealed that introduction of halogen atom on the *para*-position of phenyl of 7-diethylaminocoumarin-based 1,3,4-oxadiazole derivatives could enhance their activities. Molecular docking study suggested that **4 g** possessed an optimal docking pose with interactions inside AChE.



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KEYWORDS
Coumarin; 1,3,4-oxadiazole; acetylcholinesterase inhibitor; molecular docking

1. Introduction

Alzheimer's disease (AD) is a major age-related progressive neurodegenerative disease characterised by the destruction of nerve cells, the rapid deterioration of memory and

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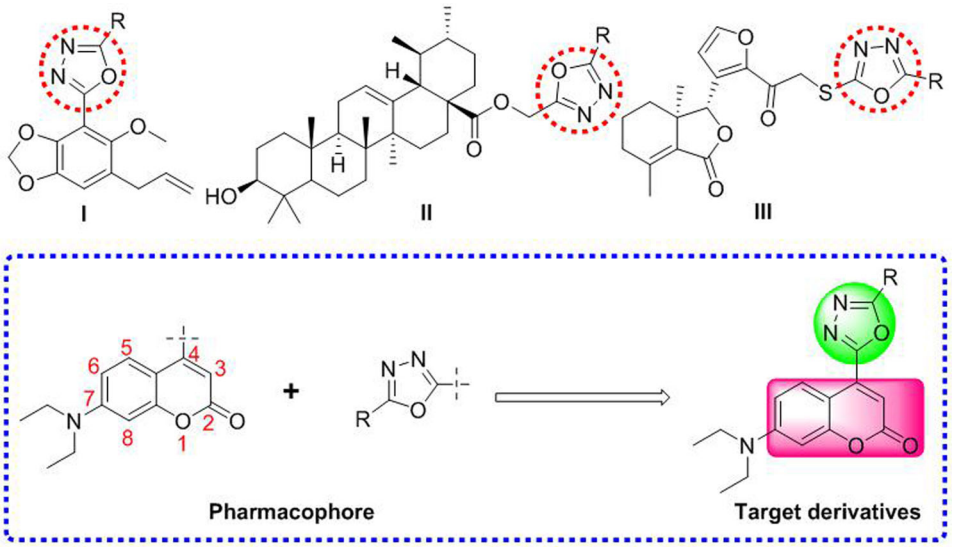
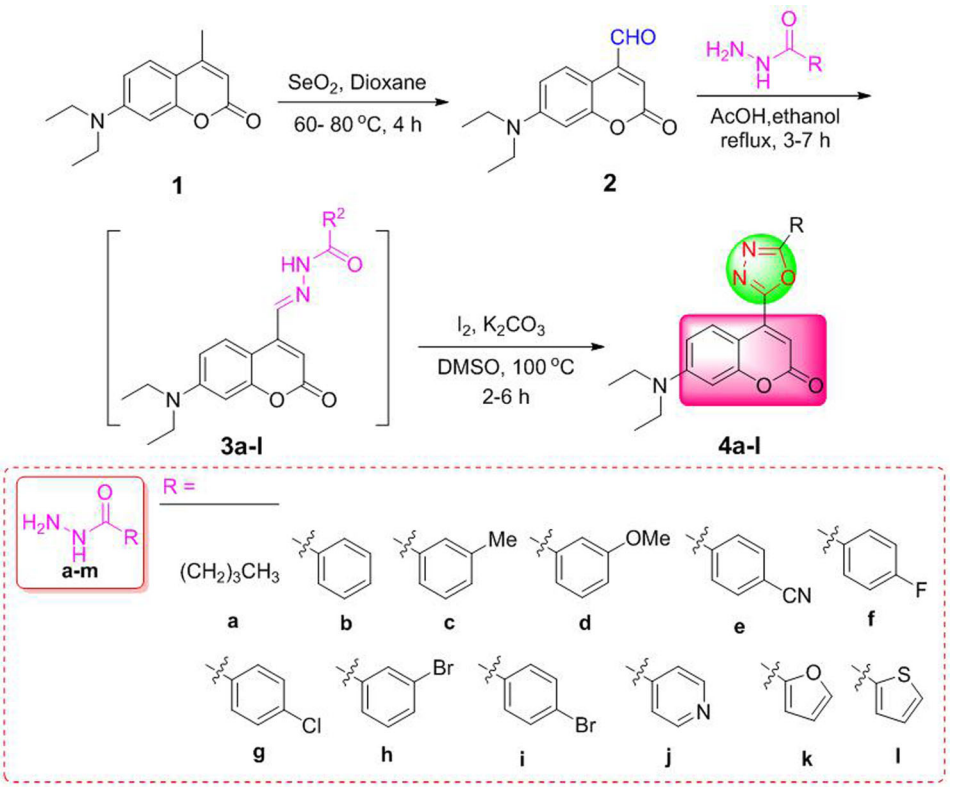


Figure 1. Structures of compounds **I-III** and the design of target derivatives.

other important cognitive functions, not only harms the health of patients, but also brings a heavy burden to their families and society [1]. It is caused by an imbalance between β-amyloid (Aβ) production and clearance, resulting in increased amount of Aβ in various forms of brain [2]. Currently, the effective treatment for AD is inhibiting brain acetylcholinesterase to increase lifetime of cerebral acetylcholine [3]. AChE, enzymes that terminate cholinergic neurotransmission in the brain, act by catalysing the hydrolysis of acetylcholine, so their inhibition can be used to alleviate memory and cognitive deficits in AD [4,5]. Therefore, the development of highly effective AChE inhibitors has been an object of research.

Coumarins, which are heterocyclic compounds with benzopyranone, commonly occur in various biologically active natural products and synthetic compounds [6,7]. It was found that coumarins showed various interesting biological activities, including anticancer, antimicrobial, antiviral, anti-inflammatory, antioxidant and other biological activities [8–11]. Recently, researchers had also reported that coumarin-based derivatives had a significant role in the studies of AChE inhibitors [12]. In addition, 1,3,4-oxadiazoles, another important class of *N*-heterocyclic compounds with a wide range of biological activities such as anticancer, antimicrobial, antiviral, analgesic activities, were widely used in medicine and pesticides [13–15]. Several attempts introducing hydrazone 1,3,4-oxadiazole moiety into natural products, such as sarisan (**I**, Figure 1) [16], ursolic acid (**II**, Figure 1) [17] and fraxinellone (**III**, Figure 1) [18], had been made to improve their biological activity. Encouraged by the above-mentioned interesting results, in this paper, we prepared a series of novel 7-diethylamino-coumarin-based 1,3,4-oxadiazole derivatives by introduction of 1,3,4-oxadiazole moiety into the coumarin skeleton. Besides, we have determined the anti-AChE activity of the target compounds by the method of Ellman and explored the possible mechanisms via molecular modeling.



Scheme 1. Synthetic route of 7-diethylaminocoumarin-based 1,3,4-oxadiazole derivatives (**4a-l**).

2. Results and discussion

2.1. Chemistry

The synthesis of coumarin-based 1,3,4-oxadiazole derivatives was shown in Scheme 1 via the method reported in some literatures [16,18]. Oxidation of 7-diethylamino-4-methylcoumarin (**1**) with SeO_2 gave 4-formyl-7-diethylaminocoumarin (**2**). Subsequently, compound **2** reacted with different arylhydrazide to afford the intermediates (**3a-l**) in the presence of HOAc. After the second reaction was completed, the solvent was evaporated under reduced pressure, and the products were not purified to save time and reagents, redissolved directly in dimethyl sulfoxide, followed by the iodine-mediated oxidative cyclisation at refluxing to obtain twelve target compounds containing 1,3,4-oxadiazole (**4a-l**). In order to prove whether the unpurified intermediates could synthesize the target compounds, some individual intermediates were purified and identified (see Supplementary Material). Comparison of ^{1}H NMR spectra of the intermediate **3j** and the target compound **4j** revealed that the protons of H-1′ (δ, 8.53 ppm) and H-3′ (δ, 12.48 ppm) disappeared proving that this step without purification was feasible. All target compounds were characterized by mp, ^{1}H NMR ^{13}C NMRand HRMS (see Supplementary Materia).

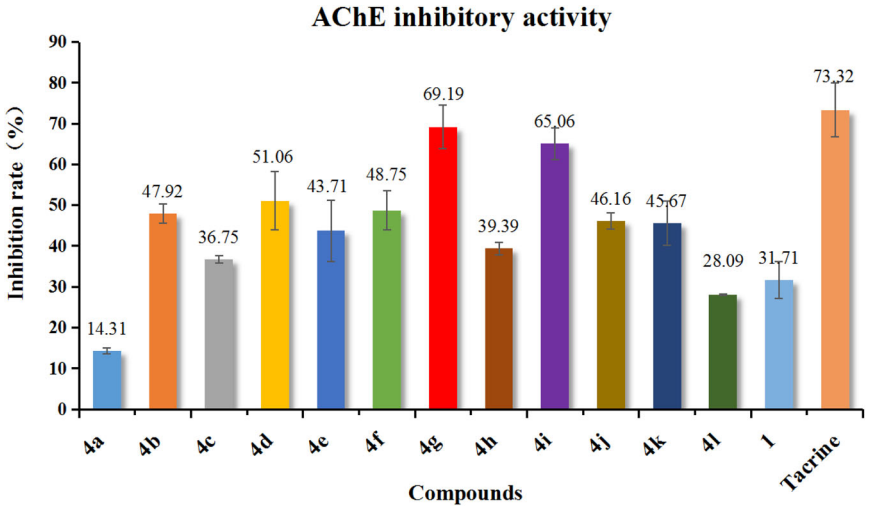


Figure 2. The AChE inhibitory activities of titled compounds (**4a-l**) at 1 μmol/ml; Values represent means ± SD; Tacrine was control and tested in 5×10^{-4} μmol/ml.

2.2. Biological activity

As shown in Figure 2, preliminary bioassay of these derivatives' activities inhibiting AChE was tested *in vitro* by method of Ellman at the concentration of 1 μmol/ml . It was apparent from this figure that most of the target compounds have better inhibitory activities against AChE compared with raw materials **1**, especially **4 g** and **4i** with an inhibitory rate of 69.19% and 65.06%, respectively, but did not exceed the positive controls. Meanwhile, analysis of the structure-activity relationship of these coumarin derivatives containing 1,3,4-thiadiazole moiety was also observed. Introduction of halogen atom on the *para*-position of phenyl of 7-diethylaminocoumarin-based 1,3,4-oxadiazole derivatives had higher activity (e.g., **4f**, **4 g** and **4i**) in comparison with the parent compound **1**. On the contrary, no significant assistance in introduction of alkyl group was found to increase activity (e.g., 14.31% for **4a**). In addition, when pyridine and furan heterocycle were introduced on 1,3,4-oxadiazole, the compounds could increase the inhibitory activity appropriately. For examlpe, the inhibition ratios of compounds **4j** and **4k** were 46.16% and 45.67%, respectively.

In order to find the lowest concentration of potent compounds, the inhibitory activities of compounds **4d**, **4 g** and **4i** with inhibition rates higher than 50% were further evaluated at concentrations of 1 μmol/ml, 0.1 μmol/ml and 0.01 μmol/ml, respectively. It could be seen from the data in Figure 3 that the inhibitory activities of the compounds gradually weaken as the concentration decreased. When the concentration reached 0.01 μmol/ml, the three compounds showed little inhibitory activities. It demonstrated that the inhibitory activities were positively correlated with the concentration of the compounds.

2.3. Molecular modeling

Molecular modeling studies of the possible binding mode of compound **4 g** in the active site of AChE were also performed to explore the possible inhibition mechanism

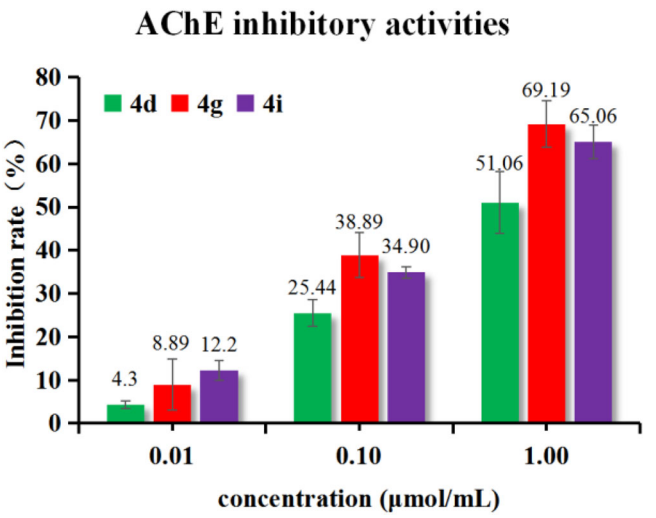


Figure 3. The AChE inhibitory activities of compounds **4d**, **4 g**, **4i** in three concentrations; Values represent means ± SD.

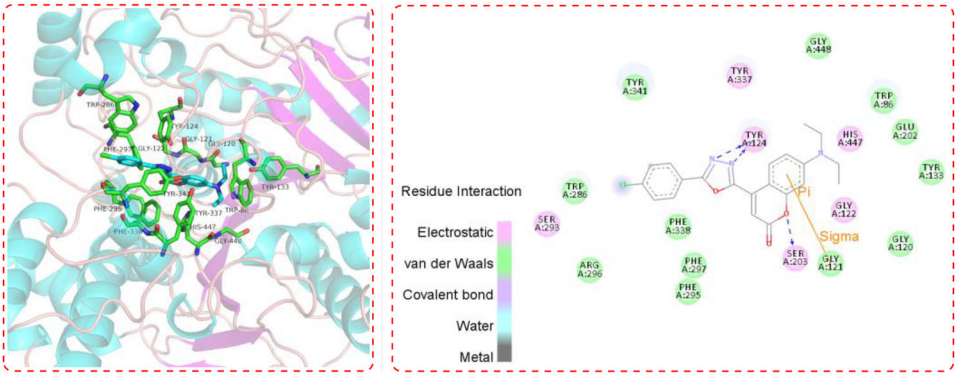


Figure 4. Docking pose of compound **4 g** inside AChE. (**a:** The 3 D structure of the compound binding model with AChE, the blue structure represents the derivative, the green structures represent amino acid residues. **b**: The 2 D structure of the compound binding model with AChE, the blue dotted lines show the hydrogen bonds, the full lines show the C-H−π interactions).

of the potent compound. The 3 D structure of human AChE was selected from the RCSB database (PDB code: 3LII) for docking studies. Interestingly, as illustrated in Figure 4, the results obtained from the preliminary analysis of molecular ducking displayed that the coumarin portion of compound **4 g** was located in the substrate of the active channel of AChE, while the benzene ring with chlorine atoms was located at the channel entrance (Figure 4, **a**). Simultaneously, the two nitrogen atoms of 1,3,4-oxadiazole core of compound **4 g** interacted with Tyr124 residue, located in the middle of the catalytic active channel of AChE, via two hydrogen bonds, oxygen atom on the 1-position of coumarin core was bonded to Ser203 residue by hydrogen bonds, and the benzene ring of coumarin maked C-H—π interaction with Gly121 residue,

located in the catalytic active site of AChE (Figure 4, **b**). Through these binding actions, compound **4 g** firmly occupied the catalytic site of AChE, while the chlorine atom with a large atomic radius controlled the entrance of the catalytic channel, which prevented acetylcholine from entering the catalytic center. This molecular docking result, along with the biological assay data, suggested that compound **4 g** was a potential inhibitor of AChE.

In summary, a series of new 7-diethylaminocoumarin-based 1,3,4-oxadiazole derivatives were designed, synthesised, and evaluated for their AChE inhibitory activities *in vitro*. Among all the derivatives, **4 g** and **4i** showed moderate inhibitory activities and the inhibitory activity was positively correlated with the concentration. Furthermore, preliminary structure-activity relationships analysis revealed that the introduction of halogen atom on the *para*-position of phenyl of 7-diethylaminocoumarin-based 1,3,4-oxadiazole derivatives could lead a promising AChE inhibitor. Molecular docking study of **4 g** and AChE also suggested that compound **4 g** was a potential inhibitor of AChE.

3. Experimental

3.1. General experimental procedures

All reagents and solvents were of reagent grade or purified according to standard methods before use. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd.). Melting points were determined on a XT-4 digital melting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and are uncorrected. Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker Avance NEO 400 or 600 MHz instrument (Bruker, Bremerhaven, Germany) in $CDCl_3$ or DMSO-d_6 using TMS (tetramethylsilane) as the internal standard. Mass spectrometry (MS) was carried out with a Waters XEVO TQ-D instrument (waters, Massachusetts, USA). High-resolution mass spectrometry (HRMS) was carried out with a Xevo G2-S QTOF instrument (waters, Massachusetts, USA).

3.2. Synthesis of compound 2

A solution of 7-diethylamino-4-methylcoumarin (1 mmol, 231.3 mg) in dioxane (10 ml) was heated to 60 °C. SeO_2 (1.1 mmol, 122.1) was added to this solution. Then the temperature was increased to 80 °C. The reaction was checked by TLC. After 12-18 h, the mixture was cooled to room temperature. The precipitates were filtered off and washed with CH_2Cl_2 (20 ml). The organic phases were combined, concentrated and separated by preparative thin-layer chromatography (PTLC) to give the pure compound **2.**

3.3. General procedure for the synthesis of compounds 3a-l

A mixture of **2** (1 mmol, 245.3 mg), hydrazides or hydrazines (1 mmol), and two drops of HOAc in EtOH (10 ml) was refluxed. When the reaction was completed by checking with TLC after 3-7 h, the resulting reaction mixture was cooled to room

temperature until no more precipitate was observed. Then the solvent was removed under reduced pressure to give the intermediates (**3a-l**). The product was not purified and went straight to the next step. Compounds **3d** and **3j** were purified and characterized by ^{1}H NMR (600 MHz) and MS.

3.3.1. Compound 3d

Yield = 74%, yellow solid, mp 236-238 °C; ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm) 12.25 (s, 1H, NH), 8.53 (s, 1H, H-1′), 8.42 (d, J = 9.0 Hz, 1H, H-5), 7.47-7.54 (m, 3H, H-Ar), 7.20 (dd, J = 8.4, 2.4 Hz, 1H, H-Ar), 6.77 (dd, J = 9.0, 2.4 Hz, 1H, H-6), 6.57 (d, J = 2.4 Hz, 1H, H-8), 6.29 (s, 1H, H-3), 3.85 (s, 3H, OCH_3), 3.44 (q, J = 7.2 Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.13 (t, J = 7.2 Hz, 6H, $N(CH_2C\underline{H}_3)_2$); MS (ESI): *m/z* 416.2 $[M+Na]^+$.

3.3.2. Compound 3j

Yield = 68%, yellow solid, mp 238-241 °C; ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm) 12.48 (s, 1H, NH), 8.82 (d, J = 5.4 Hz, 2H, H-Ar), 8.53 (s, 1H, H-1′), 8.41 (d, J = 9.0 Hz, 1H, H-5), 7.85 (d, J = 6.0 Hz, 2H, H-Ar), 6.78 (dd, J = 9.0, 2.4 Hz, 1H, H-6), 6.57 (d, J = 2.4 Hz, 1H, H-8), 6.33 (s, 1H, H-3), 3.44 (q, J = 7.2 Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.13 (t, J = 7.2 Hz, 6H, $N(CH_2C\underline{H}_3)_2$); MS (ESI): *m/z* 387.0 $[M+Na]^+$.

3.4. General procedure for the preparation of 1,3,4-oxadiazole derivatives (4a-l)

The intermediates (**3a-l**) were redissolved in DMSO (10 ml), followed by addition of K_2CO_3 (3.0 mmol, 414.6 mg), iodine (1.1 mmol, 279.4 mg) in sequence. The reaction mixture was stirred at 100 °C until the reaction was completed according to TLC analysis (2-6 h). After being cooled to room temperature, the mixture was treated with saturated $Na_2S_2O_3$ (20 ml) and extracted with ethyl acetate (3 × 20 ml). The combined organic layer was washed with brine (3 × 20 ml), dried over anhydrous Na_2SO_4, filtered and evaporated. The given residue was purified by PTLC to give the target products **4a-l** in 43-71% yield. All compounds were characterized by mp, ^{1}H NMR (400 or 600 MHz) and HRMS. The data for **4a-l** were shown as follows.

3.4.1. 4-(5-Butyl-1,3,4-oxadiazol-2-yl)-7-(diethylamino)-2H-chromen-2-one (4a)

Yield = 52%, yellow solid, mp 208-210 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.67 (d, J = 9.2 Hz, 1H, H-5), 6.67 (dd, J = 9.2, 2.6 Hz, 1H, H-6), 6.63 (s, 1H, H-3), 6.55 (d, J = 2.6 Hz, 1H, H-8), 3.45 (q, J = 7.2 Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 2.99 (t, J = 7.6 Hz, 2H, $C\underline{H}_2CH_2CH_2CH_3$), 1.83-1.88 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 1.45-1.51 (m, 2H, $CH_2CH_2C\underline{H}_2CH_3$), 1.24 (t, J = 7.2 Hz, 6H, $N(CH_2C\underline{H}_3)_2$), 1.00 (t, J = 7.2 Hz, 3H, $CH_2CH_2CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 167.8, 161.6, 161.2, 157.0, 151.1, 135.4, 128.7, 109.3, 108.8, 104.0, 97.6, 44.8 × 2, 28.4, 25.0, 22.1, 13.5, 12.4 × 2; HRESIMS: *m/z* 342.1824 $[M+H]^+$ (calcd for $C_{19}H_{24}N_3O_3$, 342.1818).

3.4.2. 7-(Diethylamino)-4-(5-phenyl-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (4 b)

Yield = 68%, yellow solid, mp 205-207 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.73 (d, J = 9.2 Hz, 1H, H-5), 8.17 (d, J = 7.2 Hz, 2H, H-Ar), 7.62 (t, J = 7.2 Hz, 1H, H-Ar),

7.58 (t, $J = 7.2$ Hz, 2H, H-Ar), 6.77 (s, 1H, H-3), 6.71 (dd, $J = 9.2$, 2.6 Hz, 1H, H-6), 6.57 (d, $J = 2.6$ Hz, 1H, H-8), 3.46 (q, $J = 7.2$ Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.25 (t, $J = 7.2$ Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 165.0, 161.4, 161.2, 157.0, 151.1, 135.3, 132.5, 129.3 × 2, 128.6, 127.3 × 2, 123.0, 109.4, 108.9, 104.1, 97.7, 44.8 × 2, 12.4 × 2; HRESIMS: *m/z* 362.1511 $[M+H]^+$ (calcd for $C_{21}H_{20}N_3O_3$, 362.1505).

3.4.3. 7-(Diethylamino)-4-(5-(m-tolyl)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (4c)

Yield = 61%, yellow solid, mp 212-214 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.74 (d, $J = 9.2$ Hz, 1H, H-5), 7.98 (s, 1H, H-Ar), 7.96 (d, $J = 7.6$ Hz, 1H, H-Ar), 7.46 (t, $J = 7.6$ Hz, 1H, H-Ar), 7.42 (d, $J = 7.6$ Hz, 1H, H-Ar), 6.78 (s, 1H, H-3), 6.71 (dd, $J = 9.2$, 2.6 Hz, 1H, H-6), 6.56 (d, $J = 2.6$ Hz, 1H, H-8), 3.46 (q, $J = 7.2$ Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 2.48 (s, 3H, $C\underline{H}_3$), 1.25 (t, $J = 7.2$ Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 165.2, 161.4, 161.3, 157.0, 151.1, 139.2, 135.3, 133.3, 129.2, 128.7, 127.8, 124.5, 122.8, 109.4, 108.8, 104.0, 97.7, 44.8 × 2, 21.3, 12.4 × 2; HRESIMS: *m/z* 376.1664 $[M+H]^+$ (calcd for $C_{22}H_{22}N_3O_3$, 376.1661).

3.4.4. 7-(Diethylamino)-4-(5-(3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (4d)

Yield = 68%, yellow solid, mp 212-214 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.72 (d, $J = 9.2$ Hz, 1H, H-5), 7.74 (d, $J = 7.6$ Hz, 1H, H-Ar), 7.67 (s, 1H, H-Ar), 7.48 (t, $J = 8.0$ Hz, 1H, H-Ar), 7.13-7.15 (m, 1H, H-Ar), 6.77 (s, 1H, H-3), 6.71 (dd, $J = 9.2$, 2.6 Hz, 1H, H-6), 6.56 (d, $J = 2.6$ Hz, 1H, H-8), 3.92 (s, 3H, $OC\underline{H}_3$), 3.46 (q, $J = 7.2$ Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.25 (t, $J = 7.2$ Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 165.0, 161.4, 161.2, 160.1, 157.0, 151.1, 135.3, 130.4, 128.6, 124.1, 119.7, 119.1, 111.8, 109.4, 108.9, 104.0, 97.7, 55.6, 44.8 × 2, 12.4 × 2; HRESIMS: *m/z* 392.1617 $[M+H]^+$ (calcd for $C_{22}H_{22}N_3O_4$, 392.1610).

3.4.5. 4-(5-(4-Cyanophenyl)-1,3,4-oxadiazol-2-yl)-7-(diethylamino)-2H-chromen-2-one (4e)

Yield = 56%, yellow solid, mp 204-206 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.67 (d, $J = 9.2$ Hz, 1H, H-5), 8.30 (d, $J = 8.4$ Hz, 2H, H-Ar), 7.89 (d, $J = 8.4$ Hz, 2H, H-Ar), 6.76 (s, 1H, H-3), 6.71 (dd, $J = 9.2$, 2.6 Hz, 1H, H-6), 6.57 (d, $J = 2.6$ Hz, 1H, H-8), 3.47 (q, $J = 7.2$ Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.25 (t, $J = 7.2$ Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.4, 162.1, 161.0, 157.1, 151.3, 134.8, 133.0 × 2, 128.4, 127.8 × 2, 126.9, 117.6, 116.0, 109.5, 109.2, 103.8, 97.7, 44.8 × 2, 12.4 × 2; HRESIMS: *m/z* 387.1458 $[M+H]^+$ (calcd for $C_{22}H_{19}N_4O_3$, 387.1457).

3.4.6. 7-(Diethylamino)-4-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (4f)

Yield = 54%, yellow solid, mp 232-234 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.71 (d, $J = 9.2$ Hz, 1H, H-5), 8.18 (dd, $J = 8.8$, 5.2 Hz, 2H, H-Ar), 7.25-7.28 (m, 2H, H-Ar), 6.75 (s, 1H, H-3), 6.70 (dd, $J = 9.2$, 2.6 Hz, 1H, H-6), 6.56 (d, $J = 2.6$ Hz, 1H, H-8), 3.46 (q, $J = 7.1$ Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.25 (t, $J = 7.1$ Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 166.5, 164.2, 161.4, 161.2, 157.1, 151.2, 135.2,

129.8, 129.7, 128.6, 119.4, 116.8, 116.6, 109.4, 108.9, 104.0, 97.6, 44.8 × 2, 12.4 × 2; HRESIMS: *m/z* 380.1416 $[M+H]^+$ (calcd for $C_{21}H_{19}N_3O_3F$, 380.1410).

3.4.7. 4-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-7-(diethylamino)-2H-chromen-2-one (4 g)

Yield = 60%, yellow solid, mp 254-256 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.70 (d, J = 9.2 Hz, 1H, H-5), 8.10 (d, J = 8.5 Hz, 2H, H-Ar), 7.56 (d, J = 8.5 Hz, 2H, H-Ar), 6.75 (s, 1H, H-3), 6.70 (dd, J = 9.2, 2.6 Hz, 1H, H-6), 6.56 (d, J = 2.6 Hz, 1H, H-8), 3.46 (q, J = 7.2 Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.25 (t, J = 7.2 Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 164.2, 161.5, 161.1, 157.0, 151.2, 138.9, 135.1, 129.7 × 2, 128.6 × 3, 121.5, 109.4, 108.9, 103.9, 97.6, 44.8 × 2, 12.4 × 2; HRESIMS: *m/z* 396.1119 $[M+H]^+$ (calcd for $C_{21}H_{19}N_3O_3Cl$, 396.1115).

3.4.8. 4-(5-(3-Bromophenyl)-1,3,4-oxadiazol-2-yl)-7-(diethylamino)-2H-chromen-2-one (4 h)

Yield = 71%, yellow solid, mp 192-194 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.69 (d, J = 9.2 Hz, 1H, H-5), 8.31 (t, J = 1.6 Hz, 1H, H-Ar), 8.10 (dt, J = 7.9, 1.6 Hz, 1H, H-Ar), 7.72-7.75 (m, 1H, H-Ar), 7.46 (t, J = 7.9 Hz, 1H, H-Ar), 6.76 (s, 1H, H-3), 6.71 (dd, J = 9.2, 2.6 Hz, 1H, H-6), 6.56 (d, J = 2.6 Hz, 1H, H-8), 3.46 (q, J = 7.1 Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.25 (t, J = 7.1 Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.7, 161.7, 161.0, 157.0, 151.0, 135.5, 135.0, 130.8, 130.2, 128.6, 125.8, 124.8, 123.3, 109.6, 109.3, 104.2, 98.0, 45.0 × 2, 12.4 × 2; HRESIMS: *m/z* 440.0612 $[M+H]^+$ (calcd for $C_{21}H_{19}N_3O_3Br$, 440.0610).

3.4.9. 4-(5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)-7-(diethylamino)-2H-chromen-2-one (4i)

Yield = 66%, yellow solid, mp 220-222 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.70 (d, J = 9.2 Hz, 1H, H-5), 8.03 (d, J = 8.6 Hz, 2H, H-Ar), 7.72 (d, J = 8.6 Hz, 2H, H-Ar)), 6.75 (s, 1H, H-3)), 6.70 (dd, J = 9.2, 2.4 Hz, 1H, H-6), 6.56 (d, J = 2.4 Hz, 1H, H-8), 3.46 (q, J = 7.1 Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.25 (t, J = 7.1 Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 164.3, 161.6, 161.1, 157.1, 151.2, 135.1, 132.7 × 2, 128.7, 128.6 × 2, 127.4, 121.9, 108.4, 109.0, 103.9, 97.7, 44.8 × 2, 12.4 × 2; HRESIMS: *m/z* 440.0613 $[M+H]^+$ (calcd for $C_{21}H_{19}N_3O_3Br$, 440.0610).

3.4.10. 7-(Diethylamino)-4-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (4j)

Yield = 52%, yellow solid, mp 259-261 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.90 (d, J = 6.0 Hz, 2H, H-Ar), 8.67 (d, J = 9.2 Hz, 1H, H-5), 8.02 (d, J = 6.0 Hz, 2H, H-Ar), 6.77 (s, 1H, H-3), 6.71 (dd, J = 9.2, 2.6 Hz, 1H, H-6), 6.57 (d, J = 2.6 Hz, 1H, H-8), 3.47 (q, J = 7.1 Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 1.25 (t, J = 7.1 Hz, 6H, $N(CH_2C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.2, 162.3, 160.9, 157.1, 151.3, 151.1 × 2, 134.8, 130.1, 128.4, 120.5 × 2, 109.5, 109.3, 103.8, 97.7, 44.8 × 2, 12.4 × 2; HRESIMS: *m/z* 363.1462 $[M+H]^+$ (calcd for $C_{20}H_{19}N_4O_3$, 363.1458).

3.4.11. 7-(Diethylamino)-4-(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (4k)

Yield = 55%, yellow solid, mp 215-217 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.70 (d, J = 9.2 Hz, 1H, H-5), 7.73 (d, J = 1.7 Hz, 1H, H-Ar), 7.33 (d, J = 3.6 Hz, 1H, H-Ar), 6.75 (s, 1H, H-3), 6.70 (dd, J = 9.2, 2.6 Hz, 1H, H-6), 6.68 (dd, J = 3.6, 1.7 Hz, 1H, H-Ar), 6.56 (d, J = 2.6 Hz, 1H, H-8), 3.46 (q, J = 7.2 Hz, 4H, $N(CH_2CH_3)_2$), 1.24 (t, J = 7.2 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 161.1, 160.7, 157.7, 157.0, 151.2, 146.6, 138.7, 134.9, 128.6, 115.7, 112.5, 109.4, 109.0, 103.9, 97.6, 44.8 × 2, 12.4 × 2; HRESIMS: m/z 352.1306 $[M+H]^+$ (calcd for $C_{19}H_{18}N_3O_4$, 352.1297).

3.4.12. 7-(Diethylamino)-4-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (4 l)

Yield = 43%, yellow solid, mp 223-225 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 8.71 (d, J = 9.2 Hz, 1H, H-5), 7.90 (dd, J = 3.8, 1.2 Hz, 1H, H-Ar), 7.66 (dd, J = 5.0, 1.2 Hz, 1H, H-Ar), 7.23-7.25 (m, 1H, H-Ar), 6.74 (s, 1H, H-3), 6.70 (dd, J = 9.2, 2.6 Hz, 1H, H-6), 6.56 (d, J = 2.6 Hz, 1H, H-8), 3.46 (q, J = 7.2 Hz, 4H, $N(CH_2CH_3)_2$), 1.25 (t, J = 7.2 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 161.3, 161.2, 160.8, 157.0, 151.1, 135.0, 131.4, 131.0, 128.6, 128.5, 124.2, 109.4, 108.8, 104.0, 97.6, 44.8 × 2, 12.4 × 2; HRESIMS: m/z 368.1077 $[M+H]^+$ (calcd for $C_{19}H_{18}N_3O_3S$, 368.1069).

3.5. In vitro biological assay

The anti-AChE activity of the 7-diethylaminocoumarin-based 1,3,4-oxadiazole derivatives was determined by the method of Ellman. The *in vitro* inhibition assays of AChE from electric eel run in phosphate buffer 0.1 M, at pH 7.4. Acetylthiocholine iodide was used as substrates, and 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) was used as the chromophoric reagent. To a 96-well plate of the sample solution (10 μl), phosphate buffer solution (PBS) (40 μl, 0.1 M, pH = 7.4), DTNB (20 μl, 2.5 mM in 0.1 M PBS, pH = 8.0) and AChE solution (10 μl, 0.2 U/ml in 0.1 M PBS, pH = 7.4) were added sequentially, shaken well, and incubated at 37 °C for 10 min. Then, acetylthiocholine iodide (20 μl, 10 mM in 0.1 M PBS, pH = 7.4) was added, shaken well again, and incubated at 37 °C for 10 min. The absorbance at 405 nm of the samples was measured using a spectrophotometer, the sample solution was set to 1 μmol/ml and the experiment was repeated three times. Tacrine was used as a positive control. Inhibitory effect (%)= $[OD_0-(OD_1-OD_2)]/OD_0\times100\%$, where OD_0 is the absorbance of blank group; OD_1 is the absorbance of sample group; OD_2 is the absorbance of sample blank group.

3.6. Molecular docking

The 3 D-structure of human AChE was downloaded from the RCSB database (PDB ID: 3LII), a chain A of the structure was used for docking study. Then, Autodock Tool was used to determine the atom types and calculate the partial charges of the protein, and the pdbqt file was generated for docking.The 2 D structure of compound

4g was drawn by ChemDraw15.0, and saved as cdx file. Then, MM2 force field in Chem3D 15.0 was used to optimize the 3 D structure of the ligand. Again, Autodock Tool was used to determine the atom types and calculate the partial charges of the ligand, and pdbqt file was generated for docking. Docking of the ligand was carried out using the Autodock vina 1.1.2 program. A sphere of 20 Å around the carbonyl group of Gly122 was defined as the binding site for the ligand docking and 250 confirmations was allowed. Pymol was used to analyze the docking solutions, and the conformation of lowest affinity value was chosen for further analysis in ligplot.

Disclosure statement

No potential conflict of interest was reported by the authors.

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